

Phylogenetic position of the small Kashmir flying squirrel, *Hylopetes fimbriatus* (\equiv *Eoglaucomys fimbriatus*), in the subfamily Pteromyinae

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Abstract: The phylogenetic relationships of flying squirrels (Pteromyinae) were studied by obtaining complete sequence data from the mitochondrial cytochrome *b* gene of eight Old World and two New World flying squirrel species, with special reference to the systematic and phylogenetic status among *Hylopetes fimbriatus* (Gray, 1837) (\equiv *Eoglaucomys fimbriatus* (Gray, 1837)) from Pakistan, two *Glaucomys* Thomas, 1908 species from North America, and two *Hylopetes* Thomas, 1908 species from Southeast Asia. Phylogenetic trees supported clustering of (i) *Belomys pearsonii* (Gray, 1842), (ii) *H. fimbriatus*, the *Glaucomys* species, *Hylopetes lepidus* (Horsfield, 1823), and *Hylopetes phayrei* (Blyth, 1859), (iii) species of *Pteromys* G. Cuvier, 1800, and (iv) species of *Petaurista* Link, 1795. Early polytomic divergence among the flying squirrel genera could have taken place in the northern part of the Eurasian continent. The unclear divergence between the Old and New World flying squirrels shows that divergence among flying squirrel genera could have occurred before the formation of the Bering Strait. *Hylopetes fimbriatus* was more closely related to the two *Glaucomys* species than to *H. lepidus* or *H. phayrei*, supporting placement of the species *fimbriatus* in the monotypic genus *Eoglaucomys* Howell, 1915.

Résumé : Nous avons étudié les relations phylogénétiques des écureuils volants (Pteromyinae) à l'aide des séquences complètes du gène mitochondrial du cytochrome *b* de huit espèces d'écureuils volants de l'Ancien Monde et de deux espèces du Nouveau Monde; nous avons porté une attention particulière au statut systématique et phylogénétique d'*Hylopetes fimbriatus* (Gray, 1837) (\equiv *Eoglaucomys fimbriatus* (Gray, 1837)) du Pakistan, des deux espèces de *Glaucomys* Thomas, 1908 d'Amérique du Nord et de deux espèces d'*Hylopetes* Thomas, 1908 du sud-est de l'Asie. Les arbres phylogénétiques appuient les regroupements suivants : (i) *Belomys pearsonii* (Gray, 1842), (ii) *H. fimbriatus*, les espèces de *Glaucomys*, *Hylopetes lepidus* (Horsfield, 1823) et *Hylopetes phayrei* (Blyth, 1859), (iii) les espèces de *Pteromys* G. Cuvier, 1800 et (iv) les espèces de *Petaurista* Link, 1795. La première divergence polytomique entre les genres d'écureuils volants a pu se produire dans la partie boréale du continent eurasiatique. La divergence imprécise entre les écureuils volants de l'Ancien et du Nouveau Monde indique que la séparation des genres a pu se faire avant la formation du détroit de Béring. *Hylopetes fimbriatus*, qui a été placé dans le genre monotypique *Eoglaucomys*, est plus fortement apparenté aux deux espèces de *Glaucomys* qu'à *H. lepidus* ou *H. phayrei*, ce qui constitue un argument en faveur de sa classification dans le genre *Eoglaucomys* Howell, 1915.

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Table 1. Species of flying squirrels (subfamily Pteromyinae) examined.

Species	Common name	Collection locality	Identification No.	Accession No.
<i>Belomys pearsonii</i>	Hairy-footed flying squirrel	Nantou, Taiwan	TW06	AB126245
<i>Glaucomys sabrinus</i> *	Northern flying squirrel	North America		AF011738
<i>Glaucomys volans</i> *	Southern flying squirrel	North America		AJ389531
<i>Hylopetes fimbriatus</i> 1	Small Kashmir flying squirrel	Ayubia National Park, Pakistan	SP801	AB126246
<i>Hylopetes fimbriatus</i> 2	Small Kashmir flying squirrel	Ayubia National Park, Pakistan	SP802	AB126247
<i>Hylopetes fimbriatus</i> 3	Small Kashmir flying squirrel	Sai Nalah, Pakistan	SP805	AB126248
<i>Hylopetes lepidus</i> 1	Gray-cheeked flying squirrel	Pasoh Forest Reserve, Negeri Sembilan, Malaysia	Y4	AB126250
<i>Hylopetes lepidus</i> 2	Gray-cheeked flying squirrel	Pasoh Forest Reserve, Negeri Sembilan, Malaysia	Y6	AB126251
<i>Hylopetes phayrei</i>	Phayre's flying squirrel	Near Vientianne, Laos	M31310	AB126252
<i>Petaurista leucogenys nikkonis</i> *	Japanese giant flying squirrel	Nagano, Japan	PL12	AB092619
<i>Petaurista petaurista albiventer</i> *	Red giant flying squirrel	Ayubia National Park, Pakistan	SP800	AB092612
<i>Pteromys momonga</i>	Japanese small flying squirrel	Fukui, Japan		AB097682
<i>Pteromys volans orii</i>	Siberian flying squirrel	Hokkaido, Japan		AB097683

Note: The numbers next to species names correspond to those in Table 3 and Figure 1. Identification No. M31310 is the index number of the National Science Museum of Japan, and the other identification numbers are our private specimen numbers.

*Sequence data are from the DNA Data Bank of Japan.

Introduction

The genus *Hylopetes* Thomas, 1908 comprises ten species: *alboniger* (Hodgson, 1836), *baberi* (Blyth, 1847), *bartelsi* Chasen, 1939, *fimbriatus* (Gray, 1837), *lepidus* (Horsfield, 1823), *nigripes* (Thomas, 1893), *phayrei* (Blyth, 1859), *sipora* Chasen, 1940, *spadiceus* (Blyth, 1847), and *winstonii* (Sody, 1949) (Corbet and Hill 1992), although Nowak (1991) recognizes only eight. These species are widely distributed from the Himalayas to the Greater Sunda Islands. Externally they are similar to species of the Palearctic genus *Pteromys* G. Cuvier, 1800 and the North American genus *Glaucomys* (Corbet and Hill 1992). The classification of the small Kashmir flying squirrel (Himalayan flying squirrel), *H. fimbriatus*, which occurs in Pakistan and Afghanistan, is still a matter of controversy. This species is mainly confined to Himalayan moist temperate forests, which have a mixture of deciduous and coniferous tree species (Roberts 1997). Howell (1915) and McKenna (1962) placed this species in the monotypic genus *Eoglaucmys* Howell, 1915, suggesting that *Eoglaucmys* is closely related to the genus *Glaucomys* Thomas, 1908 on the basis of similar dental morphology. On the other hand, Ellerman (1947) included *Eoglaucmys* in *Hylopetes*. This evaluation was followed by Ellerman and Morrison-Scott (1966), McLaughlin (1967, 1984), Honacki et al. (1982), Corbet and Hill (1992), and Hoffmann et al. (1993). However, following detailed morphological examination of bacula, foot pads, musculature, and crania, Thorington et al. (1996) suggested that *Eoglaucmys* (and thus *E. fimbriatus*) should be distinguished from *Hylopetes*. In addition, on the basis of comparative wrist anatomy of flying squirrels, Thorington and Darrow (2000) proposed a close phylogenetic relationship between *Eoglaucmys* and *Glaucomys*. However, Mercer and Roth (2003) conducted nucleotide sequence analyses of the interphotoreceptor retinoid-binding protein gene (*IRBP*) and the 12S and 16S rRNA genes and revealed that *Eoglaucmys* is basal to a clade comprising five flying squirrel genera (*Glau-*

comys, *Hylopetes*, *Iomys* Thomas, 1908, *Petaurillus* Thomas, 1908, and *Petinomys* Thomas, 1908).

To confirm the phylogenetic position of *H. fimbriatus* among flying squirrels (subfamily Pteromyinae), the complete sequence of the mitochondrial cytochrome *b* gene of *H. fimbriatus* from Pakistan was compared with that of other flying squirrel species. We discuss the phylogenetic relationships among these flying squirrel genera on the basis of the phylogenetic trees constructed, with special reference to the systematic and phylogenetic status among *H. fimbriatus* (*Eoglaucmys*), *Glaucomys*, and other *Hylopetes* species from Southeast Asia.

Materials and methods

Specimens and DNA extraction

Profiles of flying squirrels examined are shown in Table 1. Specimens of *Belomys pearsonii* (Gray, 1842), *H. fimbriatus*, *H. lepidus*, *H. phayrei*, and *Pteromys momonga* Temminck, 1844 were collected from the field (Table 1). A sample of *Pteromys volans orii* (Kuroda, 1921) was provided by the Noboribetsu Bear Park, Noboribetsu, Japan. Total genomic DNA was extracted from liver or muscle tissues using the phenol – proteinase K – sodium dodecyl sulfate method (Sambrook et al. 1989).

PCR and DNA sequencing

The entire mitochondrial cytochrome *b* gene (1140 bp) of all flying squirrels except *H. lepidus* was amplified using the polymerase chain reaction (PCR) and the primer set L14724 (5'-GATATGAAAACCATCGTTG-3') and H15910 (5'-GATTTTTGGTTTACAAGACCGAG-3') (Oshida et al. 2000a). For amplification of whole cytochrome *b* gene sequences of *H. lepidus*, two primer sets were used: (1) L14724 and H15347 (5'-GATGGGTTATTGATCCTGTTTCGTG-3') and (2) L15170 (5'-ACCACGAGGACAAATATCCTTCTGAG-3') and H15910. Primer names are

concordant with the light (L) or heavy (H) strands and the 3' end-positions of the primers in the human mitochondrial DNA sequences (Anderson et al. 1981). The 50 µl of reaction mixture contained 100 ng of genomic DNA, 25 pmol/L of each primer, 200 µmol/L dNTPs, 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L MgCl₂, and 2.5 units (1 U ≈ 16.67 nkat) of recombinant *Taq* DNA polymerase (Takara Bio Inc., Japan). Amplification was carried out for 35 cycles; the program was 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min. A final extension reaction was performed at 72 °C for 10 min. The PCR products were purified with the PCR-M[®] cleanup system (Viogene, Taiwan) and directly sequenced using an automated DNA sequencer (ABI PRISM[®] 377–96 DNA Sequencer or ABI PRISM[®] 3100 Genetic Analyzer, Applied Biosystems, California). For sequencing, the PCR primers and two complementary primers, L15576 (5'-CAGAATGATACTTCCTATTTGC-3') and H15554 (5'-GCCTATGAATGCTGTGGCTAT-3'), were employed. The purification of PCR products and the sequencing were carried out by Mission Biotech Co. Ltd. (Taipei, Taiwan).

Sequence analysis

Sequence data for *Glaucomys sabrinus* (Shaw, 1801) (Demboski et al. 1998), *Glaucomys volans* (L., 1758) (Montgelard et al. 2002), *Petaurista leucogenys nikkonis* Thomas, 1905, and *Petaurista petaurista albiventer* (Gray, 1863) from the DNA Data Bank of Japan (see Table 1) were utilized for phylogenetic analysis. All trees were rooted using *Sciurus aestuans* L., 1766 (accession No. AJ389530; Montgelard et al. 2002), *Sciurus niger* L., 1758 (accession No. U10180; Wettstein et al. 1995), and *Sciurus vulgaris* L., 1758 (accession No. AJ238588; Reyes et al. 2000). Based on the *IRBP* and 12S and 16S rRNA gene sequences, Mercer and Roth (2003) demonstrated that flying squirrels formed a sister group to the clade comprising most New World tree squirrels, including *Sciurus* (L., 1758) species. This phylogenetic relationship was also supported by Steppan et al. (2004) on the basis of *c-myc* and *RAG1* sequences. Therefore, we chose *Sciurus* species as an outgroup for assessment of phylogenetic relationships among flying squirrels.

Nucleotide sequences were aligned using DNASIS (Hitachi, Tokyo). For maximum-likelihood (ML) and neighbor-joining (NJ; Saitou and Nei 1987) analyses, we used the program Modeltest 3.06 (Posada and Crandall 1998) to select the most appropriate model of molecular evolution through a hierarchical likelihood ratio test. This test chose the TN93 model of substitution (Tamura and Nei 1993) taking into account the proportion of invariable sites (0.5032) and following a gamma distribution shape parameter of 1.1441 (TN + I + G). In the rate matrix of this substitution model, the rates of substitution of one purine for another (A-G) and of one pyrimidine for another (C-T) were estimated as 4.6680 and 15.1821, respectively, for each transversion of 1.0. The ML tree was then constructed with the heuristic search algorithm with tree bisection-reconnection in PAUP* version 4.0b10 (Swofford 2001). The NJ tree was also constructed using genetic distances corrected with the TN + I + G model in PAUP* version 4.0b10. The bootstrap values (Felsenstein 1985) were derived from 200 replications in ML analysis and 5000 replica-

Table 2. Base composition bias for the cytochrome *b* gene of 10 species of flying squirrels (subfamily Pteromyinae).

Nucleotide	Codon position			
	1st	2nd	3rd	All
A	0.290	0.200	0.353	0.281
C	0.251	0.238	0.366	0.285
G	0.213	0.138	0.025	0.125
T	0.246	0.424	0.257	0.309
Bias	0.055	0.232	0.301	0.167

Note: The 10 species of flying squirrels are those listed in Table 1. Bias was calculated according to the formula of Irwin et al. (1991).

tions in NJ analysis for assessment of their branching confidence.

Results

Cytochrome *b* gene sequences of flying squirrels

The complete sequences (1140 bp) of the cytochrome *b* genes of *B. pearsonii*, *H. fimbriatus*, *H. lepidus*, *H. phayrei*, *P. momonga*, and *P. v. orii* were successfully determined and analyzed together with previously published flying squirrel species sequences (Demboski et al. 1998; Montgelard et al. 2002). In all examined flying squirrels, sequences began with the conserved initiating methionine codon ATG. The stop codon of the gene, however, differed between flying squirrels distributed in the Old World and those distributed in the New World: all Old World flying squirrels had the stop codon AGA, whereas all New World flying squirrels had TAA.

The base composition of these sequences (Table 2) was skewed toward a deficiency in guanine (12.5% G). The other three nucleotides were more balanced (30.9% T, 28.5% C, and 28.1% A). Base frequency across in-group taxa was homogeneous ($\chi^2 = 12.41$, $df = 36$, $P = 1.0$). The frequency of guanine differed greatly among the three codon positions: 21.3% at the first position, 13.8% at the second, and 2.5% at the third. The second position had more thymine (42.4%). Adenine was more abundant at the first and third positions (29.0% and 35.3%, respectively). These values are similar to those previously found in various mammalian taxa (Irwin et al. 1991; Lara et al. 1996; Lessa and Cook 1998; Martin et al. 2000). The bias of base composition calculated according to the formula of Irwin et al. (1991) was smaller at the first and second positions (0.055 and 0.232) than at the third position (0.301) (Table 2). That is, the first and second codon positions showed less variability than the third codon position, as previously reported in cytochrome *b* genes of other mammals (Irwin et al. 1991).

Uncorrected percentage sequence divergences (*p* distances) and nucleotide substitutions among cytochrome *b* gene sequences of flying squirrels are shown in Table 3. The *p* distances among the flying squirrel genera examined ranged from 15.44% to 20.35%. The *p* distances between *H. fimbriatus* from Pakistan and *H. lepidus* and *H. phayrei* from Southeast Asia were 16.67%–18.33%. Those between *H. fimbriatus* and *Glaucomys* species were 15.26%–15.70%. The *p* distances between the two *Hylopetes* species from

Table 3. Pairwise comparisons of cytochrome *b* gene sequences (1140 bp) of 13 flying squirrel specimens.

	<i>B. pearsonii</i>	<i>G. sabrinus</i>	<i>G. volans</i>	<i>H. fimbriatus</i> 1	<i>H. fimbriatus</i> 2	<i>H. fimbriatus</i> 3	<i>H. lepidus</i> 1	<i>H. lepidus</i> 2	<i>H. phayrei</i>	<i>P. leucogenys</i>	<i>P. p. albiventer</i>	<i>P. momonga</i>	<i>P. v. orii</i>
<i>B. pearsonii</i>													
<i>G. sabrinus</i>	126/73												
<i>G. volans</i>	135/76	67/5											
<i>H. fimbriatus</i> 1	130/91	114/61	120/58										
<i>H. fimbriatus</i> 2	131/91	113/61	121/58	3/0									
<i>H. fimbriatus</i> 3	128/91	115/61	121/58	8/0	7/0								
<i>H. lepidus</i> 1	139/79	128/48	140/47	128/65	129/65	125/65							
<i>H. lepidus</i> 2	141/79	129/48	139/47	129/65	130/65	126/65	7/2						
<i>H. phayrei</i>	126/84	134/59	126/62	131/78	130/78	130/78	133/43	134/43	17.81	17.81	20.35	18.95	20.00
<i>P. l. nikkonia</i>	121/79	123/66	120/69	124/75	124/75	122/75	130/74	130/72	126/77	14.39	16.84	17.54	17.54
<i>P. p. albiventer</i>	141/77	137/70	145/71	139/75	137/75	135/75	142/72	139/72	147/85	138/26	18.77	18.77	19.56
<i>P. momonga</i>	141/85	123/76	134/75	129/89	130/89	125/89	135/80	134/80	127/89	118/74	140/74	18.77	12.54
<i>P. v. orii</i>	138/82	140/73	148/72	125/84	123/84	122/84	130/80	127/80	142/86	137/63	151/67	118/25	12.54

Note: Data above the diagonal are uncorrected percentage differences (*p* distances). Data below the diagonal are the numbers of nucleotide substitutions (transitions/transversions). The numbers next to species names correspond to those in Table 1 and Figure 1.

Southeast Asia and the *Glaucmys* species were 15.44%–16.49%.

Molecular phylogenetic analysis of flying squirrels

In the ML tree constructed under the TN + I + G model (Fig. 1a), the flying squirrels were split into four clades: *B. pearsonii*; *Hylopetes* spp. and *Glaucmys* spp.; *Pteromys* spp.; and species of *Petaurista* Link, 1795. *Hylopetes fimbriatus*, the two *Hylopetes* species of Southeast Asia, and the two *Glaucmys* species grouped together with 90% bootstrap support. In this clade, *H. lepidus* and *H. phayrei* clustered together (96% bootstrap value), but *H. fimbriatus* was separated. Each of the *Glaucmys*, *Petaurista*, and *Pteromys* clades were supported by high bootstrap values (100%, 99%, and 100%, respectively). *Belomys pearsonii* formed a sister group to the clade comprising *Hylopetes* and *Glaucmys* species, but the nodal support on its phylogenetic position was low (59%). In the NJ tree constructed with TN distances (Fig. 1b), the same four clades (*B. pearsonii*; *Hylopetes* spp. and *Glaucmys* spp.; *Pteromys* spp.; and *Petaurista* spp.) were recognized; however, *B. pearsonii* did not form a sister group to the clade of *Hylopetes* and *Glaucmys* species. The relationship between *Hylopetes* and *Glaucmys* species, however, was underpinned by lower nodal support (70%). The close relationship between *H. lepidus* and *H. phayrei* was demonstrated with an 80% bootstrap value. *Hylopetes fimbriatus* was more closely related to *Glaucmys* species than to the other *Hylopetes* species, but the nodal support was low (69%).

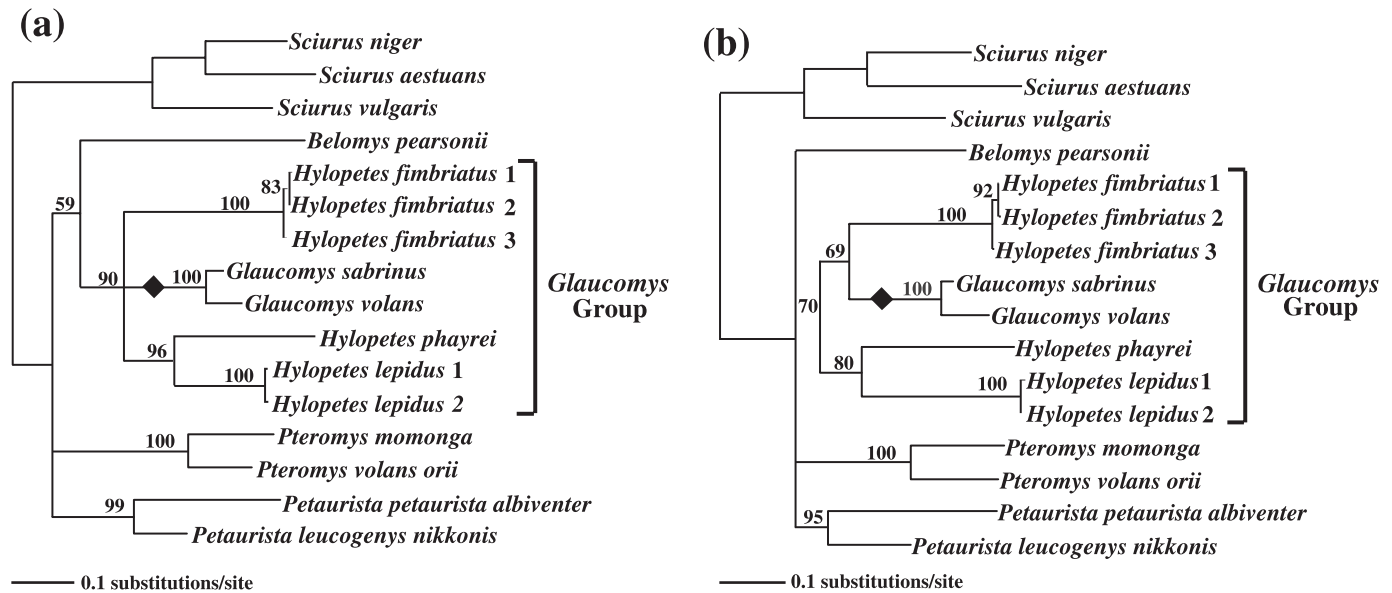
Discussion

Phylogeny of flying squirrels

The polytomic divergence of flying squirrels recognized in both phylogenetic trees indicated the presence of four major clades (Fig. 1). Although *B. pearsonii* was related to the clade comprising *Hylopetes* and *Glaucmys* species as a sister group in the ML tree, the bootstrap support was low (59%). Therefore, we recognized four polytomic clades. The clear divide between Old and New World flying squirrels isolated by the Bering Strait was not observed, suggesting that the major polytomic divergence of flying squirrels occurred before the formation of the Bering Strait. Flying squirrels are reported to be relatively abundant and diverse in European Miocene and Pliocene deposits (Mein 1970; Black 1972; De Bruijn 1999). Nine genera of flying squirrels have been defined on the basis of their fossils; seven of these occurred during the Miocene and two appeared during the Oligocene (De Bruijn 1999). Like these fossil records, analysis of partial cytochrome *b* sequences (Oshida et al. 2000b) indicates that the divergence of flying squirrels might have occurred early on the Eurasian continent. Molecular data and fossil records indicate that the primary divergence of flying squirrels may have taken place in the northern parts of Eurasia (probably in Europe). Their distribution may have expanded and shifted to southern and southeastern Asia and North America prior to further speciation.

Thorington and Darrow (2000) and Thorington et al. (2002) noted that flying squirrels could be divided into two groups on the basis of morphology: the *Glaucmys* group

Fig. 1. Phylogenetic trees of 10 flying squirrel species. The maximum-likelihood (ML) tree (a) was constructed using a heuristic search algorithm with tree bisection-reconnection, assuming the TN + I + G model of evolution. The neighbor-joining (NJ) tree (b) was constructed using genetic distances corrected with the TN + I + G model. Numbers on the branches are percentage bootstrap support values of 200 replicates for ML analysis and 5000 replicates for NJ analysis. ♦, point at which the stop codon AGA changed into TAA.



(genera *Eoglaucomys* (i.e., *H. fimbriatus*), *Glaucomys*, *Hylopetes*, *Iomys*, *Petaurillus*, and *Petinomys*) and the *Petaurista* group (genera *Aeretes* G.M. Allen, 1940, *Aeromys* Robinson and Kloss, 1915, *Belomys* Thomas, 1908, *Eupetaurus* Thomas, 1888, *Petaurista*, *Pteromys*, *Pteromyscus* Thomas, 1908, and *Trogopterus* Heude, 1898). Moreover, Mercer and Roth (2003) found this hypothesis compatible with the molecular phylogenetic evidence. The *Glaucomys* group, representing species of *Eoglaucomys* (*H. fimbriatus*), *Glaucomys*, and *Hylopetes*, was well defined in both phylogenetic trees (Fig. 1). The *p* distances within the *Glaucomys* group ranged from 15.26% to 18.33% (Table 3). On the other hand, the *Petaurista* group, comprising *B. pearsonii*, *Petaurista* spp., and *Pteromys* spp., was not formed in both trees (Fig. 1), and the *p* distances among these three genera were somewhat higher (16.84%–19.83%). The divergence within the *Petaurista* group might have occurred earlier in the evolutionary history of flying squirrels than that within the *Glaucomys* group.

It would be difficult, however, to resolve the phylogenetic relationships among flying squirrel genera by using cytochrome *b* sequences of only a few selected species. To elucidate fully the phylogenetic relationships of flying squirrels at the generic level, we need to analyze the cytochrome *b* sequences of many flying squirrel species from each flying squirrel genus.

Comparison of sequence data revealed the differences in stop codons between the Old and New World flying squirrels. Rodents show variation in the stop codon of the cytochrome *b* gene. Among muroids, for example, the subfamily Arvicolinae uses TAA for the stop codon (Martin et al. 2000). Among sciurids, AGA and TAA are used for the genera *Sciurus* (Montgelard et al. 2002) and *Tamias* Illiger, 1811 (Piaggio and Spicer 2001), respectively. We found that

the stop codon AGA was shared across all four major clades, but the TAA stop codon was found only in the two-species genus *Glaucomys*, suggesting its autapomorphic status. The stop codon could have changed just prior to the divergence of the two *Glaucomys* species.

Phylogenetic position of *Hylopetes fimbriatus* among flying squirrels

Recent wrist anatomy data (Thorington and Darrow 2000) demonstrate the close relationship between *H. fimbriatus* and the genus *Glaucomys*, confirming the classification of *H. fimbriatus* in the genus *Eoglaucomys*. In their molecular evolutionary analyses of all squirrel genera, however, Mercer and Roth (2003) demonstrated that the phylogenetic position of *Eoglaucomys* was basal to a single clade comprising *Glaucomys*, *Hylopetes*, *Iomys*, *Petaurillus*, and *Petinomys*. Oshida et al. (2000b) also reported that *Glaucomys volans* (L., 1758) was closely related to *H. phayrei* and *Petinomys setosus* (Temminck, 1844) from Southeast Asia on the basis of partial cytochrome *b* sequence data, but *H. fimbriatus* was not used in their analysis.

Compared with *p* distances of 15.26%–20.35% among flying squirrel genera, those between *H. fimbriatus* and the other *Hylopetes* species (16.67%–18.33%) were sufficiently equivalent to intergeneric values (Table 3). Assuming that *H. lepidus* and *H. phayrei* are representative forms in the genus *Hylopetes*, *H. fimbriatus* should be classified separately. Indeed, in the *Glaucomys* group, *H. fimbriatus* and the two *Hylopetes* species from Southeast Asia did not cluster together (Fig. 1). Furthermore, in the ML tree, *H. fimbriatus* was not closely related to *Glaucomys* (Fig. 1a). However, in the NJ tree, *H. fimbriatus* was most closely related to *Glaucomys*, although the bootstrap support for this branching pattern was low (69%) (Fig. 1b). Therefore, our results

support the phylogenetic hypothesis of Thorington and Darrow (2000) rather than that of Mercer and Roth (2003). From the genetic distances, *H. fimbriatus* seems to be more closely related to *G. sabrinus* than to *G. volans* (Table 3, Fig. 1b). The phylogenetic analyses of Mercer and Roth (2003) used only one species from each genus, and they chose *G. volans* to represent the genus *Glaucmys*. If Mercer and Roth (2003) had selected *G. sabrinus* instead of *G. volans*, they too may have found *H. fimbriatus* to be closely related to the *Glaucmys* species. We studied two *Glaucmys* species and three *Hylopetes* species, including *H. fimbriatus*. We did not include the other species in the *Glaucmys* group. To further clarify the phylogenetic position of *H. fimbriatus*, the molecular phylogeny and systematics of all *Hylopetes* species and all Southeast Asian members of the *Glaucmys* group should be analyzed.

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